US-PAT-NO: 7427404 in light of 6818222 B1 (**Detoxified mutants** of bacterial ADP-ribosylating toxins as parenteral **adjuvants**)

DOCUMENT-IDENTIFIER: US 7427404 B1

TITLE: Pertussis toxin mutants, bordetella strains capable of producing such mutants and their use in the development of antipertussis vaccines

DATE-ISSUED: September 23, 2008

INVENTOR-INFORMATION:

NAME	CITY	STATE ZIP CODE	COUNTRY
Pizza; Mariagrazia	Siena		IT
Covacci; Antonello	Siena		IT
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APPL-NO: 08/261691
DATE FILED: June 17, 1994

US-CL-CURRENT: **424/240.1**; **424/185.1**, **424/254.1**, **514/12**, **514/2**, **530/350**

CLAIMS:

Description Paragraph (61):
the absence of cyclodextrin, which is a major component of the culture medium, determined by thin layer chromatography. The PT mutants (80% yield) show a **purity** of 99%.

Description Paragraph (106):
Mutant PT-9K/129G purified as reported in example 3, is dialysed against (PBS), pH 7.4, containing 0.025 lysine (Ajinomoto, Japan) and 0.01% merthiolate (ELANCO, USA), for 24 hours at 4.degree. C. and then suspended again in PBS. After determination of the protein contents (Lowry, O. H. et al., (1951), J.Biol.Chem., 193:265-275), to aliquots of the mixture are different concentrations (0.035% to 0.420% w/v) of formaldehyde (4% solution in PBS, pH 7.4) so as to obtain a final ratio (**weight/weight**) mutant to formaldehyde of between 0.300 and 0.025. The resulting mixtures are incubated at 37.degree. C. for 48 hours, in the absence and in the presence of 0.025 M lysine, and then repeatedly dialysed against PBS. The mixtures are then tested to determine their free formaldehyde contents which is found to be lower than 0.01% (**weight**/volume). Furthermore, the mixture, analysed on SDS-PAGE, show the same electrophoretic pattern as PT and the presence of some extra bands one of which migrates with subunits S2 and S3 and the other, with higher molecular **weight,** with subunit S1 (FIG. 5, lane 3).

The invention claimed is:

1. Immunologically active genetically detoxified pertussis holotoxin having toxicity as measured on CHO cells of less than 0.0001 percent, and wherein the amino acid residue Glu129 (corresponding to Glu129 of FIG. 9) in the pertussis holotoxin amino acid sequence in the S1 subunit is substituted by Gly, and said S1 subunit having a substitution selected from the group consisting of: (1) Arg 9 substituted by Lys, (2) Arg13 substituted by Leu, and (3) Trp26 substituted by Ile.

2. Immunogenic formulation suitable as an acellular antipertussis vaccine for inducing in human protective immunity against infections caused by virulent Bordetella pertussis, containing an immunologically effective amount of the pertussis holotoxin of claim 1 and a pharmaceutically effective carrier.

3. Immunologically active genetically detoxified pertussis holotoxin having toxicity as measured as CHO cells of less than 0.0001 percent, and wherein the amino acid residue Glu129 (corresponding to Glu129 of FIG. 9) in the pertussis holotoxin amino acid sequence in the S1 subunit is substituted by Gly, and said S1 subunit having Arg9 substituted by Lys.

4. Immunogenic formulation suitable as an acellular antipertussis vaccine for inducing in humans protective immunity against infections caused by virulent Bordetella pertussis containing an immunologically effective amount of the pertussis holotoxin of claim 3 and a pharmaceutically effective carrier.

US Pat. 6,350,612 Pertussis toxin

APPL-NO: 07/634100
DATE FILED: December 26, 1990

PARENT-CASE:
This application is a continuation of Ser. No. 07/006,438 filed Jan. 23, 1987, which is now abandoned.

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
IT	19208/86	January 28, 1986
IT	21314/86	July 30, 1986

Brief Summary Text (27):
Recently a vaccine has been prepared which is constituted essentially by fibrous haemagglutinin (FHA) and pertussis toxin **detoxified with formaldehyde** (Sato Y., et al: Lancet Jan. 21. 122 (1984)).

11

At the end of the said period of time, the cells were separated from the culture broth by centrifuging and the pertussis toxin was recovered from the supernatant liquor by **affinity** chromotography on Affi-Gel blue (100-200 mesh) by BioRAd and on fetuin-sepharose as described by Sejura R. D. et al. [The J. Biol. Chem. 258, 23, 14647-14651 (1983)].

12

The protein obtained had a **purity** of more than 95%.

US-PAT-NO: 6818222
DOCUMENT-IDENTIFIER: US 6818222 B1

TITLE: **Detoxified mutants** of bacterial ADP-ribosylating toxins as parenteral **adjuvants**

DATE-ISSUED: November 16, 2004

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Barchfeld; Gail	Hayward	CA		
Del Giudice; Giuseppe	Siena			IT
Rappuoli; Rino				

APPL-NO: 09/044696
DATE FILED: March 18, 1998

PARENT-CASE:

CROSS-REFERENCE TO RELATED APPLICATION This application is related to provisional patent application Ser. No. 60/041,227, filed Mar. 21, 1997, from which priority is claimed under 35 USC .sctn.119(e)(1) and which is incorporated herein by reference in its entirety.

Abstract Text (1):
The present invention provides parenteral **adjuvants** comprising detoxified mutants of bacterial ADP-ribosylating toxins, particularly those from pertussis (PT), **cholera** (CT), and heat-labile E. coli (LT).

A second approach to eliminating the toxicity of CT has been to mutate the active holotoxin in order to reduce or eliminate its toxicity. The toxicity of CT resides in the A subunit and a number of **mutants** to CT and its homologue, LT, comprising point **mutations** in the A subunit, are known in the art. See, for example, International Patent Application WO92/19265. It is accepted in the art that CT and LT are generally interchangeable, showing considerable homology. ADP-ribosylating bacterial toxin **mutants** have been shown to act as mucosal **adjuvants,** see International Patent Application WO95/17211

In particularly preferred embodiments, the **non-toxic adjuvant is a detoxified mutant** selected from the group consisting of **cholera** toxin (CT), pertussis toxin (PT), and an E. coli heat-labile toxin (LT), particularly LT-K63, LT-R72, CT-S109, and PT-K9/G129.

Detailed Description Text (9):
As used herein, "**detoxified**" refers to both completely **nontoxic** and low residual toxic **mutants** of the toxin in question. Preferably, the **detoxified** protein retains a toxicity of less than 0.01% of the naturally occurring toxin counterpart, more preferably less than 0.001% and even more preferable, less than 0.0001% of the toxicity of the naturally occurring toxin counterpart. The toxicity may be measured in mouse CHO cells or preferably by evaluation of the morphological changes induced in Y1 cells. In particular, Y1 cells are adrenal tumor epithelial cells which become markedly more rounded when treated with a solution containing CT or LT (Ysamure et al., Cancer Res. (1966) 26:529-535). The toxicity of CT and LT is correlated with this morphological transition. Thus, the **mutant** toxins may be incubated with Y1 cells and the morphological changes of the cells assessed.

PROTEIN **PURIFICATION**: PRINCIPLES AND PRACTICE, Second Edition (Springer-Verlag, N.Y.), and HANDBOOK OF EXPERIMENTAL IMMUNOLOGY, VOLUMES I-IV (D. M. Weir and C. C. Blackwell eds 1986).

DOCUMENT-IDENTIFIER: US 5908825 A
** **See image for** Certificate of Correction **
TITLE: Dosage composition for nasal delivery and method of use of the same

APPL-NO: 08/781057
DATE FILED: January 9, 1997

Detailed Description Text (21):
Examples of peptide antigens which can be employed in the present invention include the B subunit of the heat-labile enterotoxin of enterotoxigenic E. coli, the B subunit of **cholera** toxin, …..

Detailed Description Text (75):
Previous studies have demonstrated that intranasal immunization with the non-toxic LT mutant LT-K63 induces a systemic response to Ova (Di Tommaso et al, Infect. Immun. 64:974-979 (1996)). LT-R72, a second LT mutant has been found to be even more immunogenic then LT-K63. However, LT-R72 has still been found to be reactogenic when tested in animal models. The mechanism by which both LT-K63 and LT-R72 induces this response has not been completely defined. However, the **molecules seem to act as a mucosal** adjuvant.

US 6149919 A
TITLE: Immunogenic **detoxified mutants of cholera** toxin and of the toxin LT, their preparation and their use for the preparation of vaccines

DATE FILED: March 25, 1997

An immunogenic **detoxified** protein comprising the amino acid sequence of subunit A of **cholera** toxin (CT-A) or subunit A of an Escherichia coli heat labile toxin (LT-A) or a fragment thereof wherein one or more amino acids at, or in positions corresponding to Val-53, Ser-63, Val-97, Tyr-104 or Pro-106 are replaced with another amino acid or deleted. Examples of specific replacements include Val-53-Asp, Val-53-Glu, Val-53-Tyr, Ser-63-Lys, Val-97-Lys, Val-97-Tyr, Tyr-104-Lys, Tyr-104-Asp, Tyr-104-Ser, Pro-106-Ser. The immunogenic **detoxified** protein is useful as vaccine for Vibrio **cholerae** or an enterotoxigenic strain of Escherichia coli and is produced by recombinant DNA means by site-directed **mutagenesis**.

Detailed Description Text (33):
By "**purified" and "isolated**" is meant, when referring to a polypeptide or nucleotide sequence, that the indicated molecule is present in the substantial absence of other biological macromolecules of the same type. The term "**purified**" as used herein preferably means at least 75% by weight, more preferably at least 85% by weight, more

preferably still at least 95% by weight, and most preferably at least 98% by weight, of biological macromolecules of the same type present (but water, buffers, and other small molecules, especially molecules having a molecular weight of less than 1000, can be present).